THE QUESTION OF CORRECT NAMING AND USE OF MICRO REAGENTS.

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All technical workers in microscopy must have found much annoyance in their experimental work. Teachers in the practical laboratories of the medical and scientific schools all testify to the great difficulty in securing reliable dyes and reagents. Few technologists are such expert chemists as they ought to be, and therefore must rely upon chemists and the manufacturers for their information. This puts the technologist at a great disadvantage, especially when he finds that the chemists do not always agree! For example, in endeavoring to show the plexus of fibrils in the nuclei of liver cells by maceration in Sulpho-Cyanide of Potassium, I met with the greatest dissatisfaction. A large local drug-house secured a sample, which I found to be the Sulpho-Cyanate of Potassium, " the same thing," I was assured. A well-known chemist was questioned, and replied, " No, they are not the same." A new sample, Merck's, had an entirely different name, yet I was told it was the article ordered. Two prominent importing chemists disagreed as to the identity of the samples and their correct names, and there the matter was dropped. If we cannot trust expert chemists to know such reagents, how can we amateurs know on whom to rely, with such a loose nomenclature?

Let us look through a text-book on Microscopy, and note the indiscriminate use of Benzol, Benzole, Benzine, Methyl Blue, Methylene Blue; Vesuvin for Bismarck Brown; Iodine Green for Methyl Green! These are serious errors, from the stand-point of solubilities, if nothing else; some dyes being soluble in water, others in alcohol. Indeed, different samples of the same anilin dye will differ in their behavior to solvents, some making a perfect solution, others leaving a more or less insoluble

residue. In some micro-books we find such statements as the following: "Iodine Green—this is another name for Methyl Green." If we are lucky enough to obtain a true, reliable Iodine green, we find the price is much higher, and the color a lighter Green. But the true dye has been largely adulterated with Methyl Green, and is now very difficult to obtain. Indeed, the terms Methyl Green, Methyl Iodide, Methyl Chloride and Methyl Bromide are applied almost indiscriminately by dealers, who substitute one for the other. For double staining Iodine Green is excellent when carefully used, and, according to Prof. H. Gibbes, a most permanent stain. It certainly does not work on the same tissues as the Methyl Green does. The differences will be found in the Proceedings of this Society for August, 1892.

Again some authors include Gentian under the head of Dahlia Violet; but if we test these dyes by their reaction to certain cells, we get different results, Methyl Violet being used for Amyloid Degeneration, while only Dahlia will show the "Mastzellen." Dahlia has a red tint, while Violet has a strong blue tint. They are both nuclear stains, but Gentain Violet requires the addition of Glacial Acetic Acid to make it directly nuclear. And we must also remember the stain mostly sold as Methyl Green is not pure, but contaminated with Methyl Violet, giving many of the reactions of the latter, as for example, with amyloid.

Amongst the many formulas for Picro-Carmine we find a variety of results which it is difficult to explain. It has always been some source of surprise to me that, when ordering a certain maker's dyes, another's should be sent out from reputable firms, either openly, as a substitution, or wrongly under a false name. These fraudulent imitations never give the results wanted. Personally, I cannot get the best counter-staining nor the true double-reaction from any other Picro-Carmine than Ranvier's. I have Hoyer's, König's and some others, and, though taking every care to follow the directions of each respective authority, have not obtained satisfactory results. It is noteworthy that the directions for using these dyes do not agree in the various manuals and text-books extant. The student, after endeavoring

to follow first one and then another, with a like poor result each time, is apt to grow skeptical with regard to the stain, and to wonder whether anyone has succeeded with it! Who does not read some article, bearing on a subject in which he has been especially interested, and wishing to verify the author's account of the extra clear demonstration of cell varieties, etc., finds out the name and sends for a sample of that particular stain or reagent? Eagerly attending to every detail, in the hope of securing exact results, he too often finds that he has had his trouble for his pains, and has succeeded only in wasting time, material and patience! Failure may arise from any one of many causes. Sometimes the author has not really meant anyone else to succeed, and has purposely omitted the designating letter or number of the dye, or some trifling modification in the technique, or the omission may arise through oversight or careless, ambiguous English! Or, again, the house from whom the reagent was ordered may send a different dye of the same name, whose chemical constitution may work havoc with the process in hand, for which it is not adapted. For instance, the use of Ammonia Alum instead of Potash Alum may seem to the theoretical histologist or pathologist of no great moment; but the technical laboratory worker knows this is not so. In making a blood stain of Alum Eosin, if Ammonia Alum be used in Alcohol, an almost solid residue is obtained, showing incompatibility, and the result for Blood is very poor if any at all. In Logwood, for Delafield's formula, he specifies Ammonia Alum—no other would do in that particular case. In Klein's Logwood, it is said, two points must be remembered, viz.: *Potash* Alum, without Ammonia, and the English Extract of Logwood, which *must* be used. When the logwood solution, as made here from the chips, becomes reddish-brown, it is usually found to contain some acid impurity which hinders staining. On the converse, in the Logwood solutions made from the continental extracts, the reddish color is nearly always obtained and gives the best results.

Eosin is said to be a blood-stain. Granted; but with the yellow, red, blue and orange varieties, some soluble in alcohol and

some in water—" which shall we buy ? " is the question. Professor Von Jaksch, I believe, is the only authority who comes forward and specifies for his particular work, by quoting in his " Chemical Diagnosis " and by giving in his lectures the name of the Eosin number and firm, and insists upon that brand. Would that we had more like him, thus saving time, money, and room in the office or laboratory.

Picro-Carmine is a dye with which it is extremely difficult to obtain the respective colors as well as the combination colors. One has too much yellow remaining, and even using the carmine will not control it; neither will the washing. Some say "Wash in alcohol after staining, as the Picric Acid is so readily soluble in water." Others say "Use acidulated water, then alcohol and then fresh alcohol." Some say "Plain water," and so forth. It depends not so much on the dye as on the maker's formula. Ranvier's has, until recently, held the chief place, and why it has been superseded is a question. Principally, I believe, it is not so readily obtained from the Garçon of the Laboratory as in years gone by, and the quality is hard to obtain just right, and we are now content with poor substitutes. Some one will say " Well, make your own dye, you have the formula in some book." The average worker with the microscope has neither time, money nor laboratory, nor, worst of all, the requisite skill and experience to make his dyes, some, as Picric Acid, being too dangerous to work with, in the hands of every one. We perhaps do turn to our book, for example "Lee's Vade Mecum," and we get a rough or not always reliable translation, or, as I said before, the minutiæ are not there. Lee carefully gives the authority, and we think we can get the original article from some reference library. "Not there" is usually the result. And this same formula, which is so indefinite, is copied into future journals and text-books without correction.

There are several kinds of Vesuvin in the market. Two kinds only are of value, the rest being useless in staining tissues. Vesuvin is freely soluble in water, while Bismarck Brown is very slightly so, hence should not be substituted for it.

Thoroughly good staining requires:

1. Fresh tissues, properly fixed and hardened.

2. Good, reliable dyes; the identical ones recommended for such use.

3. Knowledge of their acting properties, whether for direct or indirect nuclear work or for plasma and ground staining.

4. Acquaintance with their power of substitution, as Malachite Green combining with *Fuchsin* Hydrochloride; this causes the Tubercle Bacillus to lose its specific stain and color.

5. The various solubilities of the stains employed;

(a) In washing and cleaning, whether acid, neutral or alcoholic agents should be used.

(b) In mounting media, as Balsam in chloroform, etc., when we know that there are some dozen preparations of one dye on the market, differing in color, solubility and histological action, we must be careful to buy the one adapted to the case we wish to investigate; and this is not an easy matter. Grübler and Münder supply at least fourteen varieties of Safranin, and in the two houses the brands have different names and symbols. So that only the most exact ordering will insure the proper specimen. The student, directed to "stain with Safranin" may well feel at a loss to know which Safranin to use. Has microscopical technique no position, that it is placed so? Are the workers ignorant, or what is the trouble? Few microscopists are the all-round scientists that they should be, to do this work, and very few of them are so careful as they should be to note the technical side, the reaction to the tissues and the chemical combinations that result. We do not know enough of Chemistry and Physiology before we use methods of preparation. We do not always make our comparisons under similar conditions as to the action of various agents upon the same tissue. Who does not know how one agent will shrink a tissue to one-quarter its size, and another distend it to double? Still another will pick out one variety of tissue from the rest, say the mucous, only, and so on. A student is allowed a year or two of study in foundation principles, and then may take a "Major" to investigate, after doing three to

six months' Histology. He cannot give any truly reliable results, and yet it is mainly such crude work that forms the basis on which microscopy rests. To the true technologists we turn for information, but alas! how few are they, and how scattered over the world! So engaged in good work are they, and so trivial do minor points seem to them, that their reports of methods and results are brief and include only main points. We, at a distance from their laboratories, cannot tell what special dye or reagent should be bought to secure a like result, because of the unfortunate brevity, nay the *inaccuracy*, of their report.

Some firms employ an expert chemist, for economy's sake, to test and analyze their dyes. He passes a given stain on to his co-worker, the experimenting microscopist, who tests it, not only in Histology, Pathology and Bacteriology, but botanically, so as to define its best results; and as such a reagent it is then sold by the firm, every sample being thus carefully tested to secure uniform, good results. Not many years ago I was told that enormous sums were spent in buying what seemed identical dyes, but which, on testing, yielded different reactions, the firms themselves not being able to secure the same dye in every case. Anilin workers spend small fortunes in efforts to secure the true "moonlight color" for fashion purposes, as yet without success. But so great is the hope of fame and fortune, a fabric worker told me, "we are willing to try, and succeed some day." So long as fabric work holds the premier place, so long will micro work suffer, because of financial questions. Suppose a special blue is wanted—a dye is made to match the sample in shade, irrespective of the chemical combinations, provided the color is correct. It is all one to the manufacturer whether it is passed over Sulphuric, Nitric or Hydrochloric Acid; but to the microscopist such heedlessness would bring speedy disaster. In certain diseased or normal tissues, the combination of the dye with the secretions or fluids of the tissue would form a new chemical compound altogether. Indeed the chemistry of stains and their peculiar combinations with individual tissues, if fully understood, would, I believe, be of great value in throwing light on many intricate

problems in disease, by specifying the tissue in which the changes occur. This is exemplified in the case of Amyloid Degeneration, whose exact location in the vascular system is demonstrated by means of its affinity for Methyl Violet and Methyl Green. Although its histological prototype is unknown as yet, since no known normal tissue stains the same, its antecedent may yet be discovered in some of the less complex substances of the earlier degenerations, hyaline or fibroid, possibly. We should then be able to trace its development from the normal tissue, as well as its position by its specific reactions to certain dyes.

At present, perhaps the most fashionable dye in use is the Triple Stain. A curious coincidence is the indefinite mode of using the same. (Consult Lee, p. 161, 3d Edition.) There we are told "it is troublesome to prepare, but may be readily obtained prepared from Grübler. The receipt is as follows, etc.: The method given is for making from the separate dyes. Happily, Heidenhain tells us from which firm to buy, and specifies all details, as to exact name of dye, and manner of washing, and so forth. Lee says that it may be put into the hands of beginners. This is hardly correct, in my experience, and in that of many others more competent to judge. There are few stains so variable on the market. Dr. Grübler has a mixture of the three colors in the dry state. For this, you are told "to dissolve 0.4 grm of the mixture in 100 cc. of water and add 7 cc. of a 0.5 per-cent.-solution of Saurefuchsin." No further directions are given. The majority of the text-books advise after staining sections to wash in alcohol, xylol, and mount in Canada Balsam in xylol. This stain is often very hard to work with, sometimes succeeding only when the solution is old; sometimes only when freshly made. Sometimes one color predominates over the others, and we have to keep on adding and experimenting for some time. If blood covers are examined by the washing in alcohol it will be found that the corpuscles show only the Aurantia stain and what seems to be a bluish deposit of pigment which will not easily wash off. In blood-work, *water only* must be used, and that very carefully. Some find Thayer's formula by far the most

successful. The trouble with the Triple Stain, for blood, is its great fading, the indistinctness of the neutrophilic cells, especially the green staining, and the great unreliability of the stain. Possibly if some successful worker would give the precise modes of use, better results would be secured by others. The statement so frequently made that the Triple Stain is an easy, reliable one to work with ought to be disputed. Out of some 5,000 specimes made by men in London, Vienna, Berlin, Paris and the United States—mentioned in their work—showing Leukæmia, Plasmodium Malariae, tissue-sections of various kinds, Protozoa of Cancer,—out of all these only five specimens showed to any advantage, clearly and distinctly. A teacher in a medical school, eminent in scientific work, assured me he never felt certain when his work with the triple stain would be good, and that he always took the precaution to stain some covers by other methods, especially if valuable material. Not one of the many text-books published within the last three years but has the same rough-and-ready statement (copied from Lee) (?) without any remarks whatever. And yet I have four bottles of the triple dye, bought from a most reputable German firm, directly and indirectly, no two of which have the same color in powder, solution or in results! Can we wonder, then, from such instances, that so much discussion is found, especially in such high-power work as that of the Protozoan Theory for Cancers, in Bacteriology and blood-work? What is the use of publishing manuals for Clinical Diagnosis, Microscopy and the like, if modes of preparation and using are omitted? Every worker does not have the opportunity for study he would wish; but he expects the details for practical work to be clearly given in a book designed and published to aid him in some special line of investigation. He is too often wholly disappointed.

To further the microscopist's work, would it were possible one good journal could be published to cover Microscopy—or better *four*, one each for North, South, East and West. Then all matter properly belonging to the subject could be published in the journal of its own district, which would thus become a valuable

medium of communication, and save its subscribers loss of time, money and labor. Who would not infinitely prefer to subscribe to one journal devoted exclusively to the subject he is turning his attention to, instead of being compelled to pay for several journals on general medicine or scientific subjects? The single journal, when bound for reference, is a handy volume for the library, quickly consulted. With the miscellaneous scientific periodicals, one may waste hours in looking over the bulky files to find—say a vaguely remembered article on the preparation of Tubercle Bacillus in Milk. It may have been in "The American Naturalist," "Journal of Hygiene," any one of a half dozen medical journals, or in "Annals of Botany"(! !) instead of in a Journal of Microscopy, or one specially belonging to that department. Let us aim to secure concentration of the sciences, each in a proper journal of its own, carefully edited, indexed and published; instead of having, as heretofore, scattered articles, microscopical and of merit, appearing in cheap or questionable papers all over the country. Few of us are rich enough to buy many journals, or have the space to allow them to accumulate, nor yet the time to clip valuable cuttings from poor journals. Almost any one can afford *one* good journal, giving him the new methods, apparatus, bibliography, articles on his own study in each of the scientific branches in which he is especially interested. He would be glad to pay for the privilege of having his photography in a journal on photography, anatomy in an anatomical journal, and microscopy in a microscopical, more particularly in that of the National Society.